**Quantification of IAA Production by Microbes**

**Based on the colorimetric method of Gordon & Weber (1951)**

**Materials**

Day One:

125-ml flasks with 5 ml YEM +/- 0.1% tryptophan, labeled for each strain

16 strains to test (negative control is baker’s yeast, *Saccharomyces cerevisiae*)

Sterile inoculating sticks Biohazard collection bag

Alcohol burner Matches

Gloves Spray bottle of 1% bleach

Sharpie Labeling tape

Day Five:

Microfuge tubes Eppendorf rack Aluminum weigh dishes

Sharpie Vortex mixer Timer (or watch or phone)

1-ml pipetman Pipet tips Glass test tubes with reagent

Biohazard collection bag Gloves Drying oven

**Protocol**

Day One:

Sterilize your lab bench area with 0.1% bleach. Wear gloves and lab coat.

Take one set of 3 flasks and label with your name and date.

Using sterile technique, inoculate the medium with the strain for that labeled flask

Grow cultures on a shaker for 5 days

Write in your lab notebook which strain you used and whether it is in YEM or YEM with tryptophan

Day Five:

Wear gloves and lab coat

Retrieve the 3 flasks you had inoculated on Monday

Transfer 1 ml of each culture into labeled eppendorf tubes

Microfuge at maximum speed for 10’

Transfer 1 ml supernatant to glass test tubes with reagent (DANGER) for the colorimetric assay

Note the time and set timer for 25’

Measure the OD at 530 nm

Using the standard curve for IAA, calculate the amount of IAA your strain produced

We need to know the amount of IAA produced per mass of cells, so it is important to weigh the cells. To the cell pellet, add 1 ml water and vortex until completely resuspended. Transfer all of this cell suspension into labeled aluminum weigh dishes. Put into the drying oven. You will be weighing them on Monday.

**Salkowski Reagent (prepared for the class):** In the fume hood, mix 2 ml 0.5M FeCl3 and 49 ml water and 49 ml 70% perchloric acid (Oxidizer! Corrosive!).

**0.5M ferric chloride solution:** the formula/molecular weight is 270.3 so you would need 270.3 g for a liter of 1 M solution. For 10 ml, you would need 2.7g for 1 M. We want only 0.5M, so use 1.35 g in 10 ml water.

**Standard Curve:** Made standards in YEM medium at 0, 5, 10, 20, 50, and 100 µg/ml (ppm)

IAA is not soluble in water but it is in acetone

1. To a glass beaker of 10 ml acetone in the fume hood, add 10 mg IAA. Stir with metal spatula until completely dissolved. This is the 1000 µg/ml stock

2. Label a series of amber vials with the dilution series

3. Add 1 ml of the 1000 µg/ml stock to the first vial, add 9 ml medium (YEM). Mix well by inversion. This is the **100 µg/ml** standard.

4. Transfer 5 ml of the 100 µg/ml standard to another vial and add 5 ml of YEM. Mix well by inversion. This is the **50 µg/ml** standard.

5. Transfer 1 ml of the 100 µg/ml standard to another vial and add 9 ml YEM. Mix well by inversion. This is the **10 µg/ml** standard.

6. Transfer 2 ml of the 100 µg/ml standard to another vial and add 8 ml YEM. Mix well by inversion. This is the **20 µg/ml** standard.

7. Transfer 1 ml of the 50 µg/ml standard to another vial and add 9 ml YEM. Mix well by inversion. This is the **5 µg/ml** standard.

8. Transfer 2 ml of the Salkowski Reagent into 6 test tubes labeled with each standard.

9. Transfer 1 ml of each standard, including a no-IAA control of pure YEM, into the test tubes.

10. Incubate at room temperature for 25’ and then read the OD530. The differences in color across the gradient of concentrations should be evident in just a few minutes. Record the absorbance and enter into an Excel spreadsheet. Use the “scatter” function and then add trendline. Click on the options to display equation and R2 value. Solve for x and then check the equation using the standards. They should be in close agreement.

11. For the experimental values, plug into the same equation to calculate the ug/ml